

The Chemical Composition of Maple Sugar Sand^a

SUMMARY

The gritty material obtained by filtering commercial maple sirup was analyzed to determine its composition and to relate its composition to the amount of sugar sand deposited to determine the factors responsible for the formation of sugar sand. The samples, taken over a two-year period, contained calcium, malic acid, and undetermined material (probably silica) as the major constituents. The calcium, malic acid, and calcium malate content gave highly significant positive correlations with the amount of sugar sand formed, whereas the percentage of undetermined material gave a negative correlation. There was also evidence that the malic acid content was more critical in the formation of sugar sand than the calcium content. Highly significant negative correlations were obtained between the percent sugar sand deposited and the iron, copper, and boron content. Further, these constituents also gave highly significant negative correlations when compared with the calcium content of the sugar sand. The presence of potassium, magnesium, and molybdenum appeared to have little effect on the formation of sugar sand. The nonvolatile organic acids present in sugar sand were determined by paper chromatography. Results showed that sugar sand contains malic, citric, succinic, fumaric, and three unidentified organic acids.

INTRODUCTION

Sugar sand, niter, or malate of lime is an insoluble substance formed when the sap of

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D. R. Davis, formerly with the Department of Horticulture, Ohio Agricultural Experiment Station, now at Colgate Palmolive Company, New Brunswick, New Jersey. J. F. Gallander and W. A. Gould are associated with the Department of Horticulture, and J. Hacskeylo with the Department of Forestry, Ohio Agricultural Experiment Station.

the sugar maple tree (*Acer* spp.) is concentrated to maple sirup. Some of this sandy material is deposited on the evaporating pan, while most of the sugar sand stays suspended in the sirup and must be removed by sedimentation or filtration to meet federal as well as some state standards of clarity for table maple sirup.

Willits (1958) reported that the quantity and color of sugar sand are not always the same, for the constituents of the sap vary with the year, run, and sugar bush location. He also reported that the species of maple will affect the quantity of sugar sand deposited. Gallander (1961) found that sugar sand ranged in color from almost white to black, and further, these extreme color changes were noted in different runs from the same sugar bush during the same season.

Salts of organic acids are generally agreed

to be important constituents of maple sugar sand. Calcium malate has been recognized as the principal organic salt in sugar sand (Noller, 1958; Shead, 1952; Willits, 1958). Nelson (1928) separated and identified the following organic acids in maple sugar sand: l-malic, formic, acetic, fumaric, succinic, and citric acids. More recently Willits (1958) reported that maple sirup contained the following nonvolatile organic acids: malic, citric, succinic, fumaric, glycolic or dihydroxybutyric, and seven unidentified acids.

Some of the first investigators of sugar sand found varying amounts of calcium and malic acid in this material. Huston and Bryan (1899) reported that the sugar sand samples they examined contained 12.89% calcium and 20.86% malic acid. Warren (1911), in a more detailed analysis, determined some 10 constituents of sugar sand. He reported that the constituents varied only within narrow limits, and his results showed the concentration of calcium to be 17.16% and malic acid to be 51.48%. An investigation by Snell and Lockhead (1914) of six sugar sand samples that were washed and air dried showed that the insoluble material contained 65–85% calcium malate, 6–18.5% silica, minor quantities of magnesium, manganese, phosphorus, and iron, and 10–17% undetermined material.

In the present investigation, the amount of sugar sand formed in a given amount of maple sirup was related to its chemical composition. The chemical constituents were further examined and their relationship to one another and to the quantity of sugar sand deposited was studied. The results presented represent data from the analysis of sugar sand from sirups made in two successive years.

MATERIALS AND METHODS

The sugar sand samples were obtained from commercial maple sirup producers located in north-eastern Ohio. The samples from the 1960 season were from 19 producers, and the 1961 samples were from 10 producers. Each sample of sugar sand represented the deposit formed in at least 5 gallons of maple sirup. The samples were removed from the filters by scraping the sand into polyethylene bags and transporting to the Horticulture Laboratories in Wooster, where they were kept under refrigeration (32–36°F) until analyzed.

Sugar sand was arbitrarily defined for this study as the material deposited on the filter minus the moisture content and total sugar content. The percent sugar sand for each run was determined by the following formula:

$$\% \text{ SS} = 100 (WSS) / [(WMS) + (WFM)]$$

where SS = sugar sand, W = weight, MS = maple sirup, FM = material retained on the filter.

Preliminary investigations by Davis and Gallander (1959) showed that an attempt to remove the sirup from the sugar sand by washing could dissolve and remove some of the constituents of the sugar sand. All samples, therefore, were analyzed without attempting to remove the sirup portion.

The moisture content was determined on the "as received" sugar sand samples with the A.O.A.C. vacuum oven method (A.O.A.C., 1950). The sugar content was determined on the "as received" samples from both seasons and on the dried samples of the 1961 season. The sugars were inverted to reducing sugars by acid hydrolysis and determined by the A.O.A.C. procedure (A.O.A.C., 1950) and the results calculated as percent sucrose. pH was determined with a glass-electrode Beckman Zeromatic pH meter. The free acid content was determined by titrating 1.0 g of sample in 50 ml of distilled water to a pH of 8.3 with 0.01N NaOH.

The total calcium, magnesium, and potassium content of the sugar sand samples was determined by flame photometry with a Beckman DU spectrophotometer with photomultiplier and flame attachment. A 1-g dried sample was ashed at 490°C for 7 hr and then cooled and moistened with distilled water. The ash was treated with 10-ml of conc. HCl and evaporated slowly to dryness. A second 10-ml portion of conc. HCl was added, and, after again evaporating to dryness, the residue was dissolved in 20 ml of 1.5N HNO₃, transferred to a 100-ml volumetric flask, and made to volume. After the insoluble material had settled out, the solution was analyzed. The flame spectrum was measured at the following wavelengths: calcium, 422 mμ; magnesium, 285.2 mμ; and potassium, 767 mμ.

The total manganese, boron, phosphorus, molybdenum, copper, and iron content was determined by spectrographic analysis with a B & L 1.5-meter replica grating spectrograph. The samples were prepared for the analysis by ashing at 490°C for 7 hr. Absorption readings were also recorded for the lines representing aluminum and silica, and results relating to these two constituents are based on the absorption percent rather than on the actual content of aluminum or silica in the sample.

Malic acid was determined by a modification of

the method described by Willits *et al.* (1958). Since a major portion of the malic acid was present in the form of calcium malate, it was necessary to remove the calcium from the malic acid component. One g of dried sugar sand was dissolved in 125 ml of distilled water and centrifuged to remove insoluble material. The supernatant liquid was passed through a Dowex resin column (17×0.75 in.) containing 50 ml of cation resin (H^+) at a rate of 2 to 3 ml per min. The resulting effluent was diluted to 250 ml by washing the column with approximately three bed volumes of water. The remainder of the procedure was the same as that of Willits *et al.* (1958).

The total organic acids content was determined by transferring 200 ml of the cation-free sugar sand solution that was used in the malic acid determination to a Dowex 1-8X (formate) resin column (17×0.75 in.) and allowing to pass through the column at a rate of 2 ml per min. The column was washed with distilled water until the eluate gave a negative anthrone test for carbohydrate. The acids were eluted off the resin column by passing approximately 200 ml of 10N formic acid through the column at a rate of 1 to 2 ml per min. A 5-ml sample was transferred to a test tube and evaporated to dryness by passing filtered air over the surface of the liquid at $40^\circ C$. Five ml of CO_2 -free water was then added to the test tube, and the solution was titrated with 0.01N NaOH using phenolphthalein as an indicator. The percent total acids was calculated using the malic acid factor.

The presence of nonvolatile organic acids in the sugar sand samples was investigated by paper chromatographic methods. A 10-g dried sample was dissolved in 500 ml of distilled water and filtered to remove the insoluble material. Then the filtrate was passed through a Dowex 50 (hydrogen) cation exchanger. After the column was washed with 150 ml of distilled water to remove all free acids, the acids were absorbed on a Dowex 1-8X (formate) resin. This column was washed with distilled water to remove the sugars as shown by a negative anthrone test for carbohydrates. The acids were eluted from a column with 200 ml of 10N formic acid, and the effluent was concentrated by boiling until the test paper-grams would give the best resolution. The acid chromatograms were prepared by spotting on Whatman No. 1 paper and were developed by ascending chromatography with the following three solvent mixtures: I) *n*-butanol-acetic acid-water (4:1:5 v/v); II) *n*-butanol-formic acid-water (10:2:5 v/v); and III) *n*-pentanol-5N formic acid (1:1 v/v). The acids were located by spraying with a 0.04% solution of bromophenol blue in 95% ethanol at pH 6.9.

Data from the foregoing analysis were treated by statistical procedures described by Snedecor (1956).

RESULTS AND DISCUSSION

Chemical constituents causing the formation of sugar sand. Tables 1 and 2 show complete chemical analysis of sugar sand obtained from maple sirups made in 1960 and 1961. Table 3 shows the correlation coefficients of the chemical constituents as compared to the amount of sugar sand formed. Although there is a highly significant positive correlation between the amount of sugar sand formed and the percent calcium, total malic acid, total organic acids, and calcium malate for the 1960 and 1961 sirups, the correlation coefficients are not as high as was expected. The highly significant correlation between the quantity of sugar sand formed and total organic acid content is actually of little importance since in all samples the malic acid is by far the major organic acid and there is no significant correlation between the amount of sugar sand deposited and the percentage of other organic acids.

Some of the heavy metals—iron, copper, and aluminum in particular—showed significant negative correlations with the quantity of sugar sand formed. The correlation coefficients for aluminum and silica are questionable, since the amounts were not measured quantitatively, so that correlations were based only on their spectrophotometer absorption values.

Thus, the only substances that had highly significant positive correlations with the amount of sugar sand formed were calcium, total malic acid, and calcium malate. These results support the theory that calcium malate is a major constituent affecting the formation of sugar sand.

Although the presence of calcium and malic acid in the sap will, to some extent, determine the amount of sugar sand deposited, the actual amount of these two constituents (referred to as calcium malate in the tables) in the sugar sand as it comes from the filter is less than 50% (Tables 1 and 2). The sugar content of these samples, as shown in Table 2, was at least 30% in all samples, and in some it amounted to more

than 80%. Most maple sirup producers agree that as the season progresses, the amount of sugar sand formed increases. If this is true, the percentage of calcium and malic acid would also be expected to increase accordingly. Table 4, however, shows that the probability of having more sugar sand deposited in the last run as compared to the first run was very small in the 1960 samples and highly probable for those samples taken from the 1961 sirups. Further, in the samples from the 1961 season, although the amount of sugar sand formed in sirups made from the last run of sap was significantly higher than that in sirup from the first run of sap and the malic acid content also showed the same trend, the difference in the calcium content between the two runs of sirup was not significant. These results indicate that the amount of malic acid contained in the sap could be more critical than the calcium content in determining the quantity of sugar sand formed during the evaporation of the sap to sirup. The data in Tables 1 and 2 show that the ratio of calcium content to malic acid content averages about 1:3, which

Table 1. Results of the chemical analysis of sugar sand (1960).

Sample code ^a	% sugar sand in run	% Ca	% Mg	% K	% total malic acid	% total organic acids	% acids other than malic	% calcium malate ^b
031	0.06	1.52	0.171	0.625	1.67	1.71	0.04	3.23
032	0.03	0.98	0.160	0.261	2.36	2.63	0.27	3.34
045	1.20	6.81	0.047	0.289	17.70	17.92	0.22	24.51
101	0.16	0.35	0.001	0.325	2.37	2.46	0.09	2.32
102	1.09	4.60	0.044	0.258	13.94	14.56	0.62	18.54
103	0.66	3.30	0.044	0.300	10.37	10.95	0.58	13.67
161	0.23	0.35	0.103	0.300	1.44	1.52	0.08	1.79
162	0.02	0.45	0.074	0.400	1.44	1.54	0.10	1.89
163	0.02	0.45	0.056	0.355	1.15	1.89	0.74	1.60
171	0.16	5.90	0.041	0.205	15.34	15.96	0.62	21.24
172	0.35	5.78	0.044	0.350	15.11	15.84	0.73	20.89
173	0.51	7.58	0.038	0.300	22.30	23.66	1.36	11.15
181	0.16	0.55	0.017	0.375	3.38	3.40	0.02	3.93
191	0.09	1.10	0.029	0.273	4.76	4.87	0.11	5.86
192	0.54	7.13	0.065	0.210	17.91	29.62	11.71	25.04
193	0.44	5.78	0.032	0.238	17.85	18.02	0.17	23.63
213	0.71	6.90	0.019	0.213	25.18	25.34	0.16	32.08
231	0.09	0.35	0.006	0.270	1.07	1.98	0.91	1.42
241	0.01	0.98	0.087	0.233	2.31	2.78	0.47	3.29
291	0.12	0.48	0.006	0.335	0.71	1.32	0.61	1.19
302	0.03	0.35	0.000	0.270	1.11	1.45	0.34	1.46
311	0.04	0.30	0.001	0.188	0.51	0.96	0.45	0.81
312	0.01	0.30	0.015	0.083	0.48	0.84	0.36	0.78
313	0.02	0.35	0.023	0.215	0.74	1.60	0.86	1.09
351	0.08	5.25	0.047	0.335	14.45	20.48	6.03	19.70
352	0.19	7.80	0.032	0.250	19.93	26.87	6.94	27.73
371	0.42	6.60	0.044	0.305	17.42	21.93	4.51	24.02
381	0.18	0.83	0.026	0.325	3.42	4.77	1.35	4.25
411	1.30	5.90	0.001	0.253	21.02	23.14	2.12	26.92
413	1.11	5.50	0.023	0.335	16.81	17.42	0.61	22.31
445	0.40	0.65	0.006	0.353	1.34	2.34	1.00	1.99
491	0.02	1.00	0.012	0.121	2.61	4.08	1.47	3.61
Range of values	0.01—1.30%	0.30—7.80%	0.000—0.171%	0.083—0.625%	0.48—25.18%	0.84—29.62%	0.02—11.71%	0.78—32.08%

^a First two numbers designate the collaborator; the last number is the run number.

^b Percent calcium plus percent total malic acid.

Table 2. Results of the chemical analysis of sugar sand (1961).

Sample code *	% sugar sand in run	pH	% Ca	% K	% Mg	% Mn	% P	ppm Fe	ppm Cu	ppm B	ppm Mo	% free acid	% total malic acid	% total organic acids	% acids other than malic	% unde- termined material	% calcium malate ^b	% sugars of dried samples	% sugar sand of dried samples
041	0.66	6.80	3.75	0.148	0.018	0.16	0.08	347	48	5.5	0.30	0.15	8.80	9.24	0.44	12.91	12.40	73.77	26.23
046	1.42	6.85	6.41	0.148	0.025	0.18	0.20	169	21	8.1	0.79	0.13	17.20	19.82	2.62	10.91	23.48	62.31	37.69
051	0.28	7.20	0.61	0.205	0.025	0.17	0.32	677	123	8.8	0.30	0.07	0.76	0.81	0.05	17.20	1.30	80.66	19.34
057	0.64	6.80	5.45	0.158	0.018	0.16	0.10	178	11	3.8	0.30	0.16	14.25	14.51	0.26	13.25	19.54	66.35	33.65
121	0.06	7.00	0.54	0.210	0.014	0.14	0.16	520	82	6.4	0.25	0.15	1.20	1.63	0.43	11.57	1.59	85.74	14.26
125	0.05	6.80	0.72	0.213	0.011	0.19	0.31	830	65	8.3	0.79	0.20	1.43	1.74	0.31	14.68	1.95	82.14	17.86
171	0.42	6.85	6.15	0.241	0.022	0.11	0.18	1250	37	6.3	0.30	0.11	18.40	19.29	0.89	11.44	24.44	62.57	37.43
173	0.43	6.30	4.97	0.229	0.025	0.11	0.07	630	10	6.3	0.40	0.27	12.42	12.50	0.08	12.19	17.12	69.97	30.03
192	0.44	7.05	4.50	0.310	0.029	0.29	0.68	747	75	7.6	0.78	0.16	13.70	14.21	0.51	15.47	18.04	64.51	35.49
193	0.59	7.00	5.05	0.320	0.029	0.27	0.48	453	38	7.6	0.70	0.17	14.75	15.64	0.89	13.82	19.63	64.39	35.61
271	0.07	7.15	1.69	0.197	0.190	0.22	1.18	1025	143	23.0	0.88	0.13	2.85	2.97	0.12	34.16	4.41	59.39	40.61
272	0.77	6.75	10.91	0.146	0.039	0.16	0.57	254	52	8.3	0.30	0.37	38.87	40.68	1.81	13.59	49.41	33.91	66.09
281	0.36	6.70	6.35	0.380	0.018	0.07	0.12	67	15	6.8	0.17	0.12	17.56	19.05	1.49	8.28	23.79	65.80	34.20
283	0.54	6.75	5.20	0.320	0.025	0.06	0.03	38	7	5.5	0.17	0.10	15.93	16.52	0.59	6.94	21.03	70.99	29.01
361	0.12	6.80	3.95	0.245	0.018	0.12	0.48	213	41	5.0	2.46	0.27	11.06	12.01	0.95	13.10	14.74	70.08	29.92
362	0.64	6.85	4.87	0.280	0.014	0.07	0.19	138	16	5.1	0.17	0.23	13.94	15.33	1.39	8.32	18.58	70.99	29.01
411	0.41	7.15	5.25	0.224	0.036	0.07	0.24	148	15	4.6	0.40	0.12	15.94	16.22	0.28	8.39	21.07	69.73	30.27
413	0.98	7.00	4.96	0.237	0.029	0.09	0.22	178	15	3.4	0.30	0.15	14.50	14.58	0.08	8.86	19.31	71.11	28.89
431	0.18	6.90	1.69	0.270	0.036	0.07	0.16	860	40	7.2	0.17	0.20	7.56	9.30	1.74	13.00	9.05	75.54	24.46
434	0.26	6.60	3.03	0.250	0.025	0.06	0.17	788	35	5.5	0.30	0.29	10.31	11.44	1.13	11.47	13.03	73.61	26.36
Range of Values :																			
0.05—		6.30—	0.61—	0.146—	0.011—	0.06—	0.03—	38—	7—	3.4—	0.17—	0.07—	0.76—	0.81—	0.08—	6.94—	1.30—	33.90—	14.26—
1.42%		7.20	10.91%	0.380%	0.190%	0.29%	1.18%	1250	143	23	2.46	0.37%	38.87%	40.68%	2.62%	34.16%	49.41%	85.74%	66.09%
		pH																	

* First two numbers designate the collaborator; the last number is the run number.

^b Percent calcium plus percent total malic acid minus percent free acid.

Table 3. Correlation coefficients between various chemical constituents and the amount of sugar sand formed (1960 and 1961).

Constituents correlated	Correlation coefficients	
	1960	1961
% sugar sand vs:		
% calcium	.629 **	.639 **
% potassium	-.049	-.263
% magnesium	-.192	-.219
% manganese		.044
% phosphorus		-.238
ppm iron		-.513 *
ppm copper		-.483 *
ppm boron		-.287
ppm molybdenum		-.195
aluminum content		-.535 **
silica content		-.414
% calcium malate	.689 **	.584 **
pH		-.087
% free acid		-.038
% total malic acid	.689 **	.562 **
% total organic acids	.611 **	.573 **
% acids, other than malic	.062	.434
% undetermined material		-.365

* Indicates significance at the 5% level.

** Shows significance at the 1% level.

further indicates that the amount of malic acid present could be a limiting factor in the formation of calcium malate.

The chemical composition of sugar sand. Table 2 shows the percentages of the various constituents of the dried samples from the 1962 season, which contained sugar sand and adhering sugar. Variations in the total sugar content of the samples caused differences in the sugar sand content, and since the adhering sugar could not be removed before analysis without the removal of some of the sugar sand constituents, this variation was mathematically eliminated by adjusting

the values shown in Table 2 and expressing them on a sugar-free basis. This was done by dividing the percent of each constituent shown in Table 2 by the percent sugar sand of the dried sample and multiplying by 100. The adjusted values are given in Table 5.

These results are similar to those in Table 2 in that although there is a wide range of values for each constituent, the major components were calcium, malic acid, and undetermined material.

The correlation coefficients between the adjusted amount of chemical constituents and the percent sugar sand, shown in Table 6, were similar to those reported in Table 3 for the unadjusted values except that the negative correlations between iron, copper, boron, and the undetermined material become highly significant. The negative correlation coefficient between iron content and percent sugar sand ($-.629$) is higher than the positive correlation coefficient between malic acid content and percent sugar sand ($.597$) and is almost as high as the correlation coefficient between calcium content and percent sugar sand ($.655$).

Although there was no significant correlation between percent sugar sand and percent undetermined material before the values were adjusted (Table 3), after the values were adjusted to eliminate the effect of total sugars content there was a highly significant negative correlation ($-.630$). Since it has been previously reported (Snell and Lockhead, 1914) that the silica content in sugar sand is quite high, it could be assumed that most of this undetermined material is probably silica. The presence of silica in the sap apparently has little importance in the formation of sugar sand since the correlation is negative. This assumption was fur

Table 4. Statistical comparison of various constituents in sugar sand between the first run of the season and the last run of the season.

	N	First run \bar{x}	Last run \bar{x}	D.F.	t	P
1960						
Amount of sugar sand deposited	8	0.27	0.37	7	1.14	.30
1961						
Amount of sugar sand deposited	10	0.30	0.63	9	3.63	.01
Calcium content	10	3.45	5.16	9	1.43	.20
Total malic acid content	10	9.78	15.41	9	3.66	.01

Table 5. Adjusted results of the chemical analysis of sugar sand (1961).

Sample code ^a	% sugar sand in run	% Ca	% K	% Mg	% Mn	% P	ppm Fe	ppm Cu	ppm B	ppm Mo	% free acid	% total malic acid	% total organic acids	% acids other than malic	% unde- termined material	% calcium malate ^b
041	0.66	14.29	0.564	0.069	0.610	0.305	1323	183	21	1.1	0.57	33.55	35.23	1.68	48.93	47.27
046	1.42	17.01	0.393	0.066	0.478	0.531	448	56	21	2.1	0.34	45.64	52.59	6.95	28.93	62.31
051	0.28	3.15	1.060	0.129	0.879	1.654	3449	636	46	1.6	0.36	3.93	4.19	0.26	88.94	6.72
057	0.64	16.20	0.470	0.053	0.475	0.297	529	33	11	0.9	0.48	42.35	60.62	18.27	21.89	58.07
121	0.06	3.79	1.473	0.098	0.982	1.122	3647	575	45	1.8	1.05	8.42	11.43	3.01	81.11	11.16
125	0.05	4.03	1.193	0.062	1.064	1.736	4647	364	46	4.4	1.12	8.01	9.74	1.73	82.18	10.92
171	0.42	16.43	0.644	0.059	0.294	0.481	3340	99	17	0.8	0.29	49.16	51.54	2.38	30.55	65.30
173	0.43	16.55	0.763	0.083	0.366	0.233	2098	33	21	1.3	0.90	41.36	41.63	0.27	40.38	57.01
192	0.44	12.70	0.873	0.082	0.817	1.916	2105	211	21	2.2	0.45	36.80	40.04	1.44	43.57	49.05
193	0.59	14.18	0.899	0.081	0.758	1.348	1272	107	21	2.0	0.48	41.42	43.92	2.50	38.81	55.12
271	0.07	4.16	0.485	0.468	0.542	2.906	2524	352	57	2.2	0.32	7.02	7.31	0.29	84.13	10.86
272	0.77	16.51	0.220	0.059	0.242	0.862	384	80	13	0.5	0.56	58.81	61.55	4.74	20.56	74.76
281	0.36	18.57	1.111	0.053	0.205	0.351	196	44	20	0.5	0.35	51.35	55.70	4.35	24.01	69.57
283	0.54	17.92	1.103	0.086	0.207	0.103	131	24	19	0.6	0.34	54.91	56.95	2.04	23.63	72.49
361	0.12	13.20	0.819	0.060	0.401	1.604	712	137	17	8.2	0.90	36.97	40.14	3.17	43.78	49.27
362	0.64	16.79	0.965	0.048	0.241	0.655	476	55	18	0.6	0.79	48.05	52.84	4.79	28.46	64.05
411	0.41	17.29	0.738	0.119	0.230	0.790	487	49	15	1.3	0.40	52.49	53.41	0.92	27.42	69.38
413	0.98	17.17	0.820	0.100	0.312	0.762	616	52	12	1.0	0.52	50.19	50.47	0.28	30.37	66.84
431	0.18	6.91	1.104	0.147	0.286	0.654	3516	164	29	0.7	0.82	30.91	38.02	7.11	52.88	37.00
434	0.26	11.48	0.947	0.095	0.277	0.644	2986	133	21	1.1	1.10	39.07	43.35	4.28	43.21	49.45
Range of values:																
0.05 —	3.15 —	0.220 —	0.048 —	0.205 —	0.103 —	131 —	24 —	11 —	0.5 —	0.5 —	0.29 —	3.93 —	4.19 —	0.26 —	20.56 —	6.72 —
1.42%	18.57%	1.473%	0.468%	1.064%	2.906%	4647	575	57	8.2	8.2	1.12%	54.91%	56.95%	18.27%	88.94%	74.76%

^a First two numbers designate the collaborator; the last number is the run number.^b Percent calcium plus percent total malic acid minus percent free acid.

ther supported by Gallander (1961), who analyzed the sap from a cooperator that has had no sugar sand formation in the past 15 years and found that silica was present in concentrations equivalent to that of other cooperators who had the usual sugar sand deposits.

None of the highly significant correlation coefficients between the amount of sugar sand deposited and the various chemical constituents are particularly high. However, there is a definite indication that in those sirups in which a large amount of sugar sand was deposited, the major constituents of the sugar sand were calcium and malic acid (presumably as calcium malate). In runs in which only a small amount of sugar sand was formed, the sugar sand was composed mainly of undetermined material (probably silica and extraneous material). It

is quite possible that this deposition of undetermined material is fairly constant between runs and even between cooperators since its importance increased only as the amount of sugar sand formed in a given amount of sirup decreased.

Table 6 also shows the relation between calcium content and several of the other important constituents of sugar sand. The positive relation between calcium content and total malic acid content is highly significant (.953) and further supports the theory concerning the formation of insoluble calcium malate during evaporation. The high negative correlation between calcium content and boron content (−.908) can be partially explained by the established negative effect of calcium on the uptake of boron in plants. Experimental results concerning the highly significant negative correlation coefficients between calcium content and copper (−.903) and iron (−.818) content are still too scanty to permit a general conclusion.

The range of values of every constituent tested during both seasons is quite large (Tables 1 and 2). This is not unusual, since the sirup samples represent different areas within the state. Table 2 compares two runs from the same cooperator, showing only minor differences between the two runs of each producer in the amounts of potassium, magnesium, and manganese. The amounts of magnesium, manganese, and molybdenum in the sugar sand samples are not correlated with the amount of sugar sand deposited, and their presence apparently has no relation to the deposition of sugar sand (Tables 3 and 6).

Nonvolatile organic acids. Fig. 1 gives typical chromatograms showing the location of the organic acids in sugar sand. The *n*-pentanol-5*N* formic acid (1:1 v/v) developing solvent gave the best resolution of the three solvents used in this study. Because of the masking effect of the large amount of malic acid present, three chromatograms were made on each sugar sand sample. Each chromatogram represents the material washed from approximately one-third of a column. The nonvolatile acids were identified by relating the R_f value of each spot on the chromatogram to the R_f

Table 6. Correlation coefficients between the adjusted values of the chemical constituents and the amount of sugar sand formed and the adjusted percent calcium—1961 season.

Constituents correlated	Correlation coefficient
% sugar sand vs:	
% calcium	.655 **
% potassium	−.557 *
% magnesium	−.339
% manganese	−.292
% phosphorus	−.460 *
ppm iron	−.629 **
ppm copper	−.535 *
ppm boron	−.575 **
ppm molybdenum	−.333
% calcium malate	.620 **
% free acids	−.438
% total malic acid	.597 **
% total organic acids	.608 **
% acids, other than malic	.216
% undetermined material	−.630 **
% calcium (adjusted) vs:	
% potassium	−.428 *
% magnesium	−.496 *
% manganese	−.696 **
% phosphorus	−.678 **
ppm iron	−.818 **
ppm copper	−.903 **
ppm boron	−.908 **
ppm molybdenum	−.283
% total malic acid	.953 **

* Indicates significance at the 5% level.

** Shows significance at the 1% level.

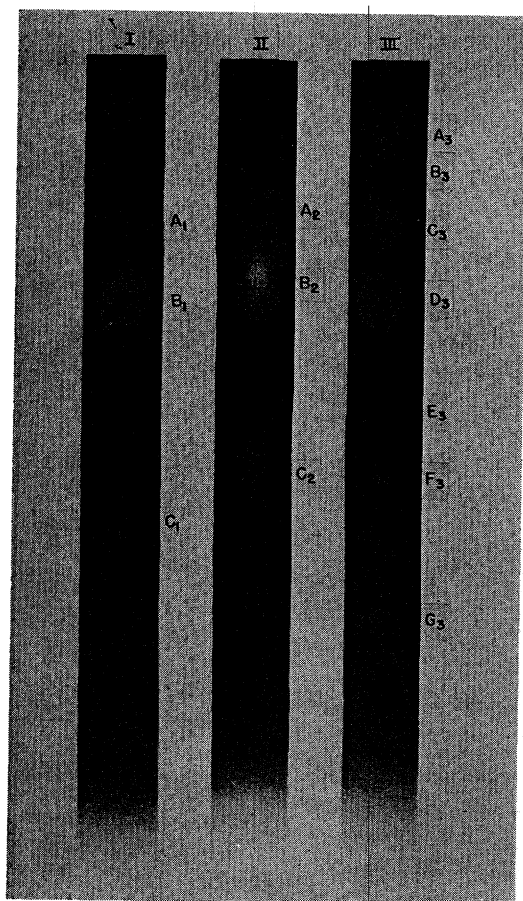


Fig. 1. Typical paper chromatograms of a sugar sand sample, showing the location of the organic acids.

values of the reference compounds. Four of the seven acids present were identified by their R_f values as citric (spots A_1 , A_2 , and C_3), malic (spots B_1 , B_2 , and D_3), succinic (spot F_3), and fumaric (spot G_3). The presence of these four acids has been reported by Nelson (1928) and Porter *et al.* (1951).

It is quite evident in Fig. 1 that the concentration of malic acid was considerably higher than that of the other acids. These

results, supported by the chemical analysis of the sugar sand shown in Tables 1 and 2, are attributed to the relatively high concentrations of calcium malate in sugar sand.

The presence of other unidentified acids in maple products was reported by Willits (1958), whose results indicated the presence of four unidentified acids in maple sap and sirup and three additional unidentified acids in the sirup.

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